In vitro Adenosine Detection with a Diamond-based Sensor

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In the central nervous system, adenosine serves as a modulator of neurotransmission and a neuroprotective agent against ischemic- and seizure-induced neuronal injury. It may be synthesized either intracellularly from degradation of adenosine monophosphate (AMP) or extracellularly by the metabolism of nucleotides. Several experimental approaches, *e.g.*, microwave irradiation or microdialysis, have been used to measure *in vitro* and *in vivo* concentrations of adenosine; a basal adenosine level was estimated at 50-200 nM[1]. However, these techniques suffer from various disadvantages, *e.g.*, inducing serious tissue damage and having low temporal resolution (minute time scale). Alternatively, adenosine is electrochemically active, and thus can be detected in real-time with electrochemical techniques including amperometry or fast-scan cyclic voltammetry (FSCV). Amperometry offers excellent temporal resolution and sensitivity, but is typically difficult to interpret for component mixtures. On the other hand, FSCV can generate a "finger print" response for each analyte, potentially allowing a distinguishable measurement in the presence of multiple components. With these methods, microelectrodes are employed, minimizing tissue damage. Recently, Brajter-Toth *et al.* used FSCV in a flow cell to detect adenosine at a carbon-fiber microelectrode, proposing a mechanism for adenosine reduction and oxidation[2]. However, the carbon-fiber electrode displayed limited sensitivity, ~10 μM. If used *in vivo* or *in vitro*, stimulation of the neuron to release substantial amount of adenosine is necessary, introducing the risk of altering the neural circuitry involved.

Boron-doped diamond electrodes are increasingly used in electroanalysis because of their exceptional electrochemical properties, including low baseline current and wide potential window of water decomposition;[3] adenosine detection provides opportunity to demonstrate both of these advantages. The diamond surface should also discourage adsorption, which likely translates into improved stability upon implantation of the device into biological systems. We are currently developing implantable, diamond-based microelectrodes to study neurotransmission in the brain and central nervous system. The diamond electrodes (**Fig.1**) mimic the needle-like geometry of widely used carbon-fiber electrodes. They are fabricated by selective CVD deposition of a diamond thin film onto a tungsten wire substrate, which is pre-sealed into quartz. With this electrodes, we have detected 10 nM adenosine using flow injection analysis.[4] With this electrode, we aim to investigate the role of adenosine in neurotransmission (ms time scale) under various physiological conditions. In this study, we focus on the PreBötzinger Complex (PreBötC) in the (rodent) brain stem, a putative site for respiratory rhythmogenesis, as a model to demonstrate the capability of a diamond-based probe as an *in vitro*, adenosine sensor.

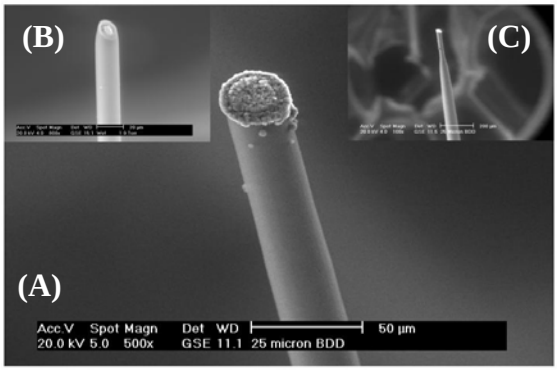

Fig. 1: *Scanning electron images of a diamond microelectrode: (A) selective diamond growth at the tip, approx. 30 μm diameter, 2~5 μm (111) crystals dominant; (B) tungsten wire substrate sealed into quartz capillary; (c) intact electrode body.*

In the PreBötC, neurons with "pacemaker" autorhythmic properties are hypothesized to generate inspiratory rhythm. The neural activity may be inhibited by the administration of adenosine or adenosine analogues during early development[5]. However, the mechanism whereby adenosine inhibits inspiratory drive is not well characterized. There are multiple adenosine receptor subtypes: A_1, A_{2A}, A_{2B}, and A_3, present in PreBötC, with different affinities for adenosine. When each receptor is activated, it can either inhibit or excite neurons. Thus, it is necessary to individually understand the effects of

these receptors on inspiratory drive. Here, we present our initial results (**Fig.2)** of *in vitro* adenosine detection from selective A_{2A} receptor activation.

A brain slice containing PreBötC was obtained from a neonatal (P1-P3) Sprague-Dawley rat. The slice was perfused with artificial cerebrospinal fluid (aCSF), saturated with 95% O_2 and 5% CO_2. Respiratory frequency and magnitude were indirectly monitored by extracellular, suction-electrode recording from hypoglossal nerve rootlets; this provides an index of inspiratory drive in young animals. The diamond microelectrode was lowered into the PreBötC, with Ag/AgCl as the reference/counter electrode. Its potential was continuously scanned between –1.0 V and +1.5 V, at 500 V/s (see **Fig. 2(A)**). After administration of CGS 21680, an adenosine analogue selective for A_{2A} receptor, several "island" features representing the occurrence of electrochemical reactions, were observed on the 3-D false-color plot (**Fig. 2(A)**). A typical cyclic voltammogram, **Fig. 2(B)**, obtained by extracting data from the color plot along the Y-axis, showed an oxidation peak near +0.8V for adenosine[2]. The peak-current for oxidation of adenosine, **Fig. 2(C)**, increased in a series of waves, whose frequencies varied between 50 and 200 seconds. After post-calibration of the diamond electrode **(Fig. 2(D))**, it was determined that the *in vitro* adenosine concentration varied between 2 to 5 µM **(Fig. 2(E))**. With respect to repiratory rhythm, the data suggest that CGS-induced adenosine release decreases the respiratory frequency and magnitude, as hypothesized (data not shown). A_{2A} activation, therefore, inhibits the inspiratory drive. Further investigation of the roles played by other receptors is in progress.

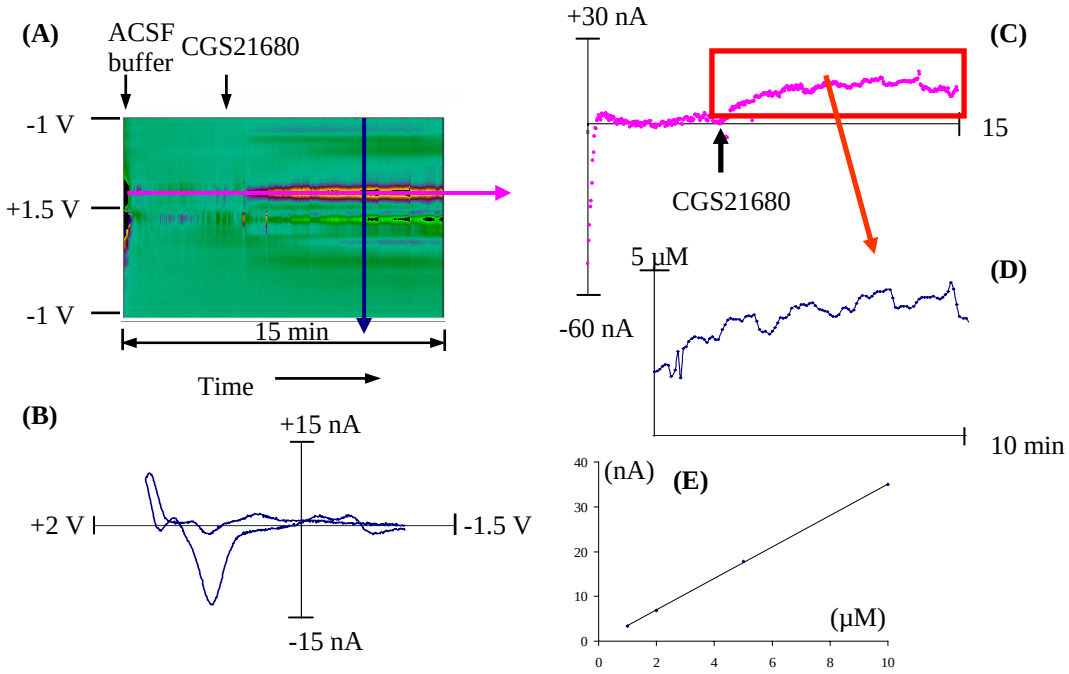

Fig. 2: *In vitro adenosine measurement; (A) 3D false color plot of released adenosine when CGS 21680 was administrated; (B) Finger print of adenosine, cyclic voltammogram; (C) Current increased after CGS 21680 treatment, which was related to the variation of adenosine concentration (D) by post calibration of electrode at flow injection system (E).*

References

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